Nuclease

Week 6

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

Definitions

BL21(DE3)

Top10

pSB1C3-Nuc

pSB1K3-T7-Nuc

PCR

ddH_2O

EcoRI

SpeI

XbaI

PstI

SDS

pSB1C3-T7

pSB1C3-T7-Nuc

T4 DNA ligase

pSB1K3

T7

1 Digestion of pSB1C3-Nuc with gel purification and PCR analysis - second attempt

Responsible

Oscar He and Ellinor Lindholm

Protocols used

3A Assembly
Q5 PCR Amplification
Gel Purification

Modifications and comments to protocols

3A Assembly:	Amount of DNA per reaction:	100 ng
	Number of double digestion duplicates:	3
	Number of single digestion duplicates:	1
	Volume of Enzyme Master Mix (MM) for each double digestion (dd) reaction:	4 μl
	Volume of Enzyme Master Mix (MM) for each single digestion (sd) reaction:	2 μl
	Total Volume of each digestion reaction:	8 μl
Q5 PCR Amplification:	1 μl DNA sample used in PCR mix.	
Q5 PCR Amplification primers:	VF2_R (reverse) VF2_F (forward)	

Experimental Setup

Table 1: Concentration of samples used for digestion.

Sample	Concentration [ng/μl]
Nuc 2.1 GS	56.3
Nuc 1 HQ	44.8

Table 2: Volumes for each component in the enzyme Master Mixes.

Component	MM EcoRI [μl]	MaM SpeI [μl]	MM XbaI [μl]	MM PstI [μl]
10x CutSmart Buffer	-	-	1.6	1.6
ddH_2O	-	-	13.6	13.6
Enzyme	-	-	0.8	0.8

Table 3: Volumes of each component used for the digestion mixes.

Component	Nuc 2.1 GS (sd) [μl]	Nuc 2.1 GS (sd) [μl]	Nuc 2.1 GS (dd) [μl]	Nuc 1 HQ (sd) [μl]	Nuc 1 HQ (sd) [μl]	Nuc 1 HQ (dd) [μl]
DNA Sample	1.78	1.78	1.78	2.23	2.23	2.23
MM XbaI	2	-	2	2	-	2
MM PstI	.	2	2	-	2	2
10x CutSmart Buffer	0.6	0.6	0.4	0.6	0.6	0.4
ddH_2O	3.62	3.62	1.82	3.17	3.17	1.37

Table 4: Weights of the cut out gel bits and volumes of Buffer QG and Iso-propanol used for the gel purification.

Sample	Weights of gel cut out [g]	Buffer QG [μl]	Iso-propanol [μl]
Nuc 2.1.1 GS dd	0.3273	981.9	327.3
Nuc 2.1.2 GS dd	0.3457	1037.1	345.7
Nuc 2.1.3 GS dd	0.3214	964.2	321.4
Nuc 2.1.1 GS dd SDS	0.1805	541.5	180.5
Nuc 2.1.2 GS dd SDS	0.186	558	186
Nuc 2.1.3 GS dd SDS	0.1591	477.3	159.1
Nuc 1.1 HQ dd	0.3493	1047.9	349.3
Nuc 1.2 HQ dd	0.2189	656.7	218.9
Nuc 1.3 HQ dd	0.2864	859.2	286.4
Nuc 1.1 HQ dd SDS	0.1878	563.4	187.8
Nuc 1.2 HQ dd SDS	0.2099	629.7	209.9
Nuc 1.3 HQ dd SDS	0.169	507	169

Sample Calculation

Volume of enzyme stock solution in each enzyme Master Mix

$$Volume\ enzyme = \frac{Amount\ of\ DNA\ per\ reaction}{1000 \cdot (Number\ of\ sd + Number\ of\ dd)} \tag{1}$$

Volume DNA sample in each digestion reaction

$$Volume\ DNA\ sample = \frac{Amount\ DNA\ per\ reaction}{Concentration\ of\ DNA\ sample} \tag{2}$$

Results and Conclusions

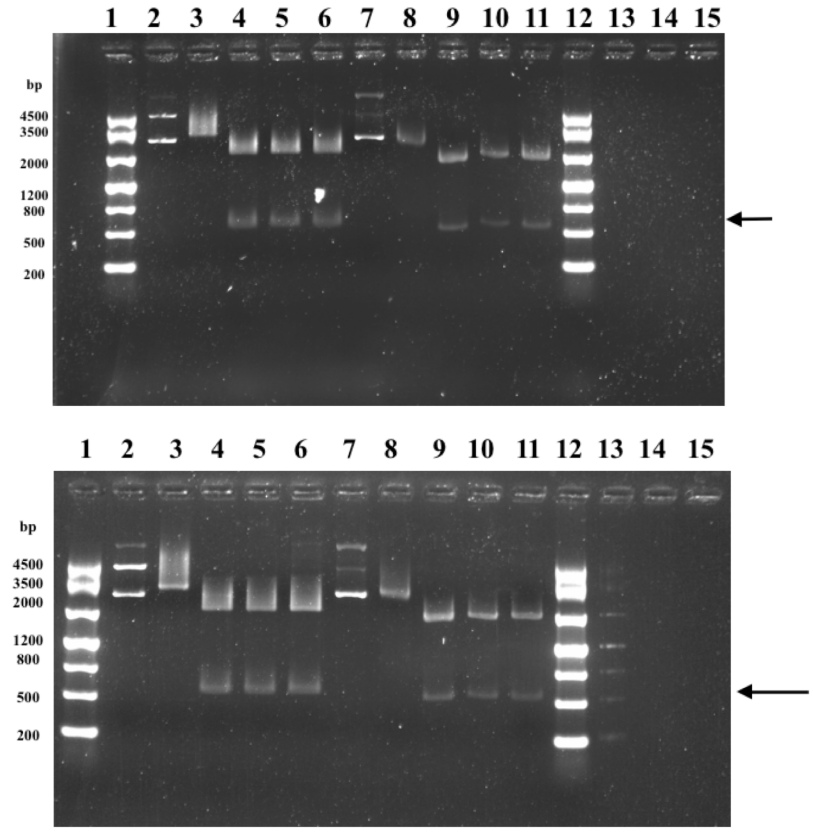

Figure 1: Result of the gel electrophoresis with 1 % agarose at 100 V. 1 % SDS is added to the samples in well 8-11 in both gels.

In bands 4, 5, and 6 as well as in bands 9, 10 and 11 there are two bands at different sizes; around 2200 bp and between 500-600 bp. The first band corresponds to a linearized backbone and the lower band to Nuc. This indicates that the digestion was successful.

Table 5: Expected size(s) and actual size(s) for the samples in the gel electrophoresis.

Well # (top)	Sample	Expected size [bp]	Actual size [bp]
1	DNA Ladder	-	-
2	Nuc 2.1.1 GS sd XbaI	2631	3400
3	Nuc 2.1.2 GS sd PstI	2631	3500
4	Nuc 2.1.1 GS dd	2070 561	2000-3000 600
5	Nuc 2.1.2 GS dd	2070 561	2000-3000 600
6	Nuc 2.1.3 GS dd	2070 561	2000-3000 600
7	Negative control	2700-2800	-
8	Nuc 2.1.1 GS sd PstI + SDS	2631	3500
9	Nuc 2.1.1 GS dd + SDS	2070 561	2000 500+
10	Nuc 2.1.2 GS dd + SDS	2070 561	2000 500+
11	Nuc 2.1.3 GS dd + SDS	2070 561	2000 500+
12	DNA Ladder	-	-
13	-	-	-
14	-	-	-
15	-	-	-
Well # (bottom)	Sample	Expected size [bp]	Actual size [bp]
1	DNA Ladder	-	-
2	Nuc 1.1 HQ sd XbaI	2631	3400
3	Nuc 1.2 HQ sd PstI	2631	3500
4	Nuc 1.1 HQ dd	2070 561	2000-3000 600
5	Nuc 1.2 HQ dd	2070 561	2000-3000 600
6	Nuc 1.3 HQ dd	2070 561	2000-3000 600
7	Negative control	2700-2800	-
8	Nuc 1.1 HQ sd PstI + SDS	2631	3500
9	Nuc 1.1 HQ dd + SDS	2070 561	2000 500+
10	Nuc 1.1 HQ dd + SDS	2070 561	2000 500+
11	Nuc 1.1 HQ dd + SDS	2070 561	2000 500+
12	DNA Ladder	-	-
13	-	-	-
14	-	-	-
15	-	-	-

Table 6: Concentrations of the gel purified samples and after pooling 1.2-2.3.

Sample	Concentration [ng/μl]
Nuc 2.1.1 GS	3.6
Nuc 2.1.2 GS	5.0
Nuc 2.1.3 GS	3.1
Nuc 2.1.1 GS SDS	3.1
Nuc 2.1.2 GS SDS	5.1
Nuc 2.1.3 GS SDS	2.3
Nuc 1.1 HQ	4.8
Nuc 1.2 HQ	2.7
Nuc 1.3 HQ	3.7
Nuc 1.1 HQ SDS	2.3
Nuc 1.2 HQ SDS	2.5
Nuc 1.3 HQ SDS	5.2

Discussion and Troubleshooting

When purifying Nuc from the digestion by cutting out the bands from the gel, the resulting concentrations are much lower than expected by this method. This could for example be due to insufficient cutting of the bands. However, the concentrations are still usable and therefor kept for further experiments.

2 3A Assembly (ligation) of pSB1K3-T7-Nuc with PCR analysis

Responsible

Oscar He and Ellinor Lindholm

Protocols used

3A Assembly
Q5 PCR Amplification

Modifications and comments to protocols

	Amount of vector per reaction	50 ng ($3.68 \cdot 10^{-14}$ mol)
	Volume of T4 DNA ligase	0.5 μl
3A Assembly:	Volume of 10x T4 DNA ligase buffer	3 μl
	Total reaction volume	20 μl
	Ratio vector:insert	1:3

Q5 PCR Amplification: 1 μl DNA sample used in PCR mix.
Q5 PCR Amplification primers: VF2_R (reverse)
VF2_F (forward)

Experimental Setup

Table 7: Concentration and size of the parts used for ligation in the 3A assembly.

Assemble part	Concentration [ng/μl]	Size [bp]
pSB1K3	4.9	2204
T7	6.6	55
Nuc 2.1.3 GS	3.1	561
Nuc 1.3 HQ SDS	5.2	561

Table 8: Volumes of each component used in the ligation mix.

Assemble part	Nuc 2.1.3 GS [μl]	Nuc 1.3 HQ SDS [μl]
pSB1K3	10.20	10.20
T7	0.52	0.52
DNA Sample	7.36	12.34
T4 DNA Ligase	0.5	0.5
10x T4 DNA Ligase Buffer	2	2
ddH_2O	0	0

Sample Calculation

Moles of vector and mass of insert per reaction were calculated with NEBioCalculator^{TM}.

Volume of pSB1A3, T7 and nuclease

$$Volume\ of\ vector = \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ vector\ sample} \tag{3}$$

$$Volume\ of\ insert = Ratio\ \cdot\ \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ insert\ sample} \tag{4}$$

Results and Conclusions

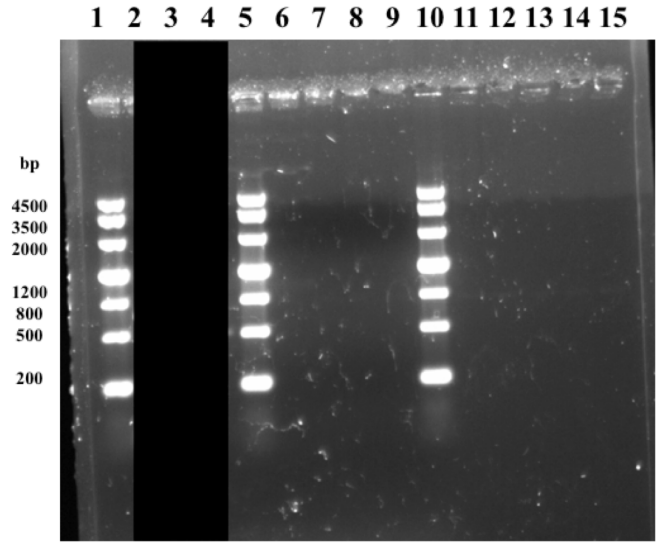

Figure 2: Result of the gel electrophoresis of the ligation product with 1 % agarose at 100 V. (1) DNA Ladder (5) DNA Ladder (6) pSB1K3-T7-Nuc 2.1.3 GS (7) pSB1K3-T7-Nuc 2.1.3 GS (8) pSB1K3-T7-Nuc 1.3 HQ SDS (9) pSB1K3-T7-Nuc 1.3 HQ SDS (10) DNA Ladder.

In the gel after ligation, (Figure 2), there are no visible bands in well 6-9.

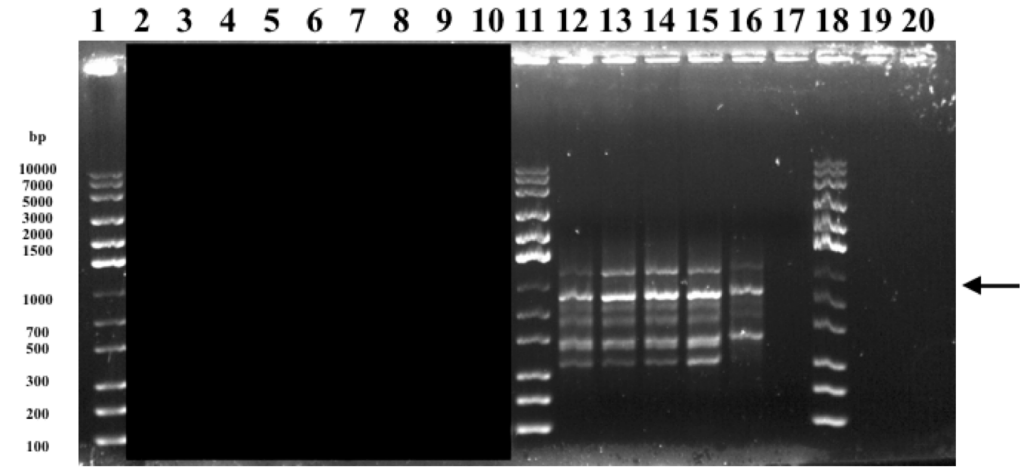

Figure 3: Result of the gel electrophoresis of PCR amplification product with 1 % agarose at 100 V. (1) DNA Ladder (11) DNA Ladder (12) pSB1K3-T7-Nuc 2.1.3 GS (13) pSB1K3-T7-Nuc 2.1.3 GS (14) pSB1K3-T7-Nuc 1.3 HQ SDS (15) pSB1K3-T7-Nuc 1.3 HQ SDS (16) Positive control (17) Negative control (18) DNA Ladder.

Discussion and Troubleshooting

Observing the gel (Figure 2), it is clear that no bands are visible. However, this does not mean that the ligation has failed as the concentration most certainly is low enough to result in too low intensities when analyzing the gel. Therefore the samples were PCR amplified to reach a higher concentration and the gel result can be seen in Figure 3. Even though the gel electrophoresis result is not optimal with a degree of smearing and with unspecific bands, the bands with the highest intensity is the wanted size with approximately 800 bp. The ligation was concluded as successful.

3 Transformation of pSB1K3-T7-Nuc in Top10 cells

Responsible

Oscar He and Ellinor Lindholm

Protocols used

Transformation

Results and Conclusions

The transformation was unsuccessful as no colonies were seen.

Discussion and Troubleshooting

There are two reasons for an unsuccessful transformation in this case. One reason could be that the ligation was unsuccessful and therefor the cells could not survive. Another reason could be that some mistakes were made when following the Transformation protocol.

4 PCR amplification of extracted pSB1C3-Nuc plasmids

Responsible

Oscar He, Ellinor Lindholm and Maren Maanja

Protocols used

Q5 PCR Amplification

Modifications and comments to protocols

Q5 PCR Amplification:	1 μl DNA sample used in PCR mix.
Q5 PCR Amplification primers:	VF2_R (reverse)
	VF2_F (forward)

Experimental Setup

Table 9: Concentration of the pSB1C3-Nuc plasmid used for PCR amplification.

Sample	Concentration [ng/μl]
Nuc 1.1 GS	51.8
Nuc 1.1 GS (10x diluted)	5.18

Results and Conclusions

The gel figure is missing.

Table 10: Concentration of the pSB1C3-Nuc PCR product, measured with NanoDrop at 280 nm.

Sample	Concentration [ng/μl]
Nuc 1.1.1 GS	33.9
Nuc 1.1.2 GS	42.6

Discussion and Troubleshooting

The concentration of the PCR products were not high enough to use in further experiments.

5 Digestion of pSB1C3-Nuc PCR Amplification product and pSB1C3-T7 with gel purification and PCR analysis

Responsible

Maren Maanja

Protocols used

3A Assembly
Q5 PCR Amplification
Gel Purification

Modifications and comments to protocols

3A Assembly:	Amount of DNA per reaction:	100 ng
	Number of double digestion duplicates:	3
	Number of single digestion duplicates:	1
	Volume of Enzyme Master Mix (MM) for each double digestion (dd) reaction:	4 μl
	Volume of Enzyme Master Mix (MM) for each single digestion (sd) reaction:	2 μl
	Total Volume of each digestion reaction:	8 μl
Q5 PCR Amplification:	1 μl DNA sample used in PCR mix.	
Q5 PCR Amplification primers:	VF2_R (reverse) VF2_F (forward)	

Experimental Setup

Table 11: Concentration of samples used for digestion.

Sample	Concentration [ng/μl]
Nuc 1.1.2 GS	42.6
pSB1C3-T7	292.5

Table 12: Volumes for each component in the enzyme Master Mixes.

Component	MM EcoRI [μl]	MaM SpeI [μl]	MM XbaI [μl]	MM PstI [μl]
10x CutSmart Buffer	0.8	0.8	0.8	0.8
ddH_2O	6.8	6.8	6.8	6.8
Enzyme	0.4	0.4	0.4	0.4

Table 13: Volumes of each component used for the digestion mixes.

Component	Nuc 1.1.2 GS (sd) [μl]	Nuc 1.1.2 GS (sd) [μl]	Nuc 1.1.2 GS (dd) [μl]	pSB1C3-T7 (sd) [μl]	pSB1C3-T7 (sd) [μl]	pSB1C3-T7 (dd) [μl]
DNA Sample	2.35	2.35	2.35	0.34	0.34	0.34
MM EcoRI	-	-	-	2	-	2
MM SpeI	-	-	-	-	2	2
MM XbaI	2	-	2	-	-	-
MM PstI	-	2	2	-	-	-
10x CutSmart Buffer	0.6	0.6	0.4	0.6	0.6	0.4
ddH2O	5.06	5.06	3.26	3.05	3.05	1.25

Sample Calculation

Volume of enzyme stock solution in each enzyme Master Mix

$$Volume\ enzyme = \frac{Amount\ of\ DNA\ per\ reaction}{1000 \cdot (Number\ of\ sd + Number\ of\ dd)} \tag{5}$$

Volume DNA sample in each digestion reaction

$$Volume\ DNA\ sample = \frac{Amount\ DNA\ per\ reaction}{Concentration\ of\ DNA\ sample} \tag{6}$$

Results and Conclusions

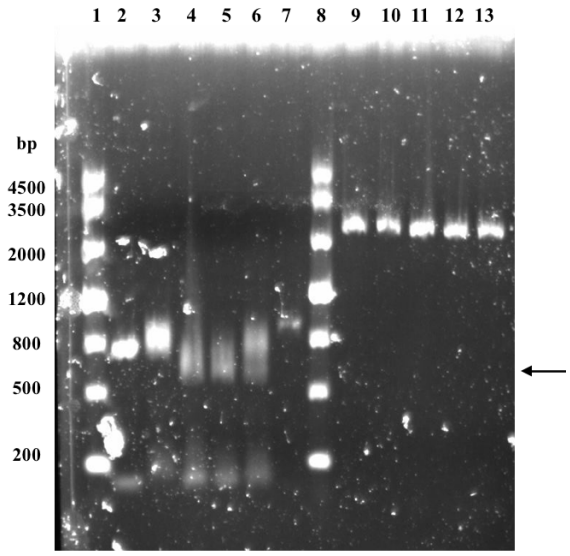

Figure 4: Result of the gel electrophoresis of the digestion product with 1 % agarose at 100 V. (1) DNA Ladder (2) Nuc 1.1.2 GS sd XbaI (3) Nuc 1.1.2 GS sd PstI (4) Nuc 1.1.2.1 GS dd (5) Nuc 1.1.2.2 GS dd (6) Nuc 1.1.2.3 GS dd (7) Negative control (8) DNA Ladder (9) pSB1C3-T7 sd EcoRI (10) pSB1C3-T7 sd SpeI (11) pSB1C3-T7 dd (12) pSB1C3-T7 dd (13) pSB1C3-T7 dd (14) Negative control (15) DNA Ladder.

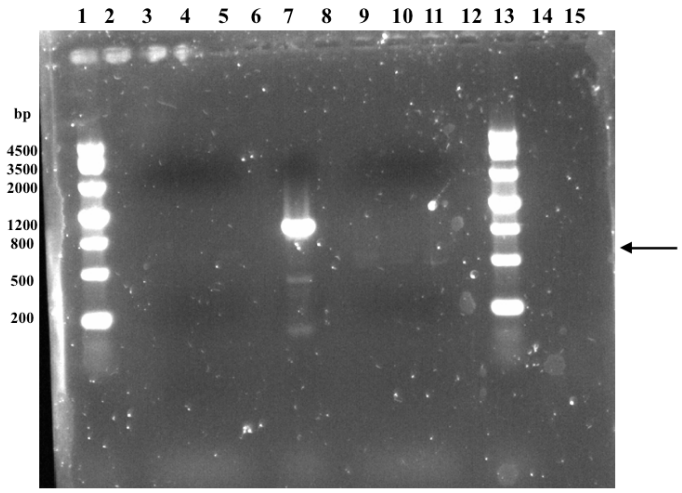

Figure 5: Result of the gel electrophoresis of the gel purified product with 1 % agarose at 100 V. (1) DNA Ladder (2) Nuc 1.1.2.1 GS dd (3) Nuc 1.1.2.2 GS dd (4) Nuc 1.1.2.3 GS dd (5) Negative control (6) Nuc 1.1.2.1 GS dd SDS (7) Nuc 1.1.2.2 GS dd SDS (8) Nuc 1.1.2.3 GS dd SDS (9) DNA Ladder.

Table 14: The concentration of the sample after digestion, measured with NanoDrop at 280 nm.

Sample	Concentration [ng/μl]
Nuc 1.1.2.1 GS dd	2.2
Nuc 1.1.2.2 GS dd	2.1
Nuc 1.1.2.3 GS dd	N/A

The bands in well 4, 5 and 6 (see Figure 5) have the right size of the digested PCR product and the digestion was assumed to be successful. Faint bands can also be seen in the gel with the PCR purified product in Figure 14.

Discussion and Troubleshooting

The final concentrations of the purified, digested Nuc samples are very low. This is probably due to major losses in the gel purification procedure as seen by separate tests. However, the samples can still be used for ligation even though the ratios most probably will be undesirably low.

6 3A Assembly (ligation) of pSB1K3-T7-Nuc

Responsible

Oscar He and Ellinor Lindholm

Protocols used

3A Assembly

Modifications and comments to protocols

	Amount of vector per reaction	50 ng ($3.68 \cdot 10^{-14}$ mol)
	Volume of T4 DNA ligase	0.5 μl
3A Assembly:	Volume of 10x T4 DNA ligase buffer	2 μl
	Total reaction volume	20 μl
	Ratio vector:insert	1:3

Experimental Setup

Table 15: Concentration and size of the parts used for ligation in the 3A assembly.

Assemble part	Concentration [ng/μl]	Size [bp]
pSB1K3	4.9	2204
T7	6.6	55
Nuc 2.1.2 GS SDS	5.1	561
Nuc 1.2 HQ	2.7	561

Table 16: Volumes of each component used in the ligation mix.

Assemble part	Nuc 2.1.2 GS SDS [μl]	Nuc 1.2 HQ [μl]
pSB1K3	10.20	10.20
T7	0.52	0.52
DNA Sample	7.5	14.17
T4 DNA Ligase	0.5	0.5
10x T4 DNA Ligase Buffer	2	2
ddH_2O	0	0

Sample Calculation

Moles of vector and mass of insert per reaction were calculated with NEBioCalculator^{TM}.

Volume of pSB1A3, T7 and nuclease

$$Volume\ of\ vector = \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ vector\ sample} \tag{7}$$

$$Volume\ of\ insert = Ratio\ \cdot\ \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ insert\ sample} \tag{8}$$

Results and Conclusions

The ligation will be proven successful by analyzing the results after the transformation. A successful ligation, assuming transformation conditions are valid, means the cells can grow.

Discussion and Troubleshooting

For this ligation, the concentrations used are very low. This is something that can result in an unsuccessful ligation as the conditions are not optimal.

7 Transformation of pSB1K3-T7-Nuc in Top10 and BL21(DE3) cells and inoculation in liquid media

Responsible

Oscar He and Ellinor Lindholm

Protocols used

Transformation

Results and Conclusions

The transformation was successful since colonies were present after incubation.

Discussion and Troubleshooting

As the transformation was successful it was concluded that the ligation must have been successful and the ligation product could be proceeded to be purified from the cells.

8 Extraction of pSB1K3-T7-Nuc from transformed Top10 cells with PCR analysis

Responsible

Oscar He and Ellinor Lindholm

Protocols used

Plasmid Extraction
Q5 PCR Amplification

Modifications and comments to protocols

DNA Extraction (Qiagen) - Step 1:	centrifugation at 13,000 rpm ($\sim$ 17,000 x g)
DNA Extraction (Qiagen) - Step 7:	not performed for some samples (marked "no PB")
DNA Extraction (Qiagen) - Step 10:	DNA elution using Buffer EB
Q5 PCR Amplification:	1 μl DNA sample used in PCR mix.
Q5 PCR Amplification primers:	VF2_R (reverse)
	VF2_F (forward)

Experimental Setup

Table 17: Volumes of each liquid cell over night culture used for DNA plasmid extraction.

Sample	Volumes [μl]
Nuc Top10 1	5
Nuc Top10 2	5
Nuc BL21(DE3) 1	5
Nuc BL21(DE3) 2	5
Nuc BL21(DE3) 3	5
Nuc BL21(DE3) 4	5
Nuc BL21(DE3) 1 No PB	5
Nuc BL21(DE3) 2 No PB	5
Nuc BL21(DE3) 3 No PB	5
Nuc BL21(DE3) 4 No PB	5

Table 18: Calculated concentration of sample plasmid in PCR mix.

Sample	Concentration [ng/μl]
Nuc Top10 1	0.44
Nuc Top10 1.1	0.504
Nuc Top10 1 no PB	0.836
Nuc TOP10 2	0.78
Nuc Top10 2.1	0.592
Nuc Top10 2 no PB	1.152

Sample Calculation

The final concentration of plasmid sample in PCR mix

$$Final\ concentration = \frac{Initial\ concentration}{Dilution\ factor} \tag{9}$$

Concentration of sample Nuc BL21(DE3) 1

$$\frac{96.3}{25} = 3.852 \approx 3.9\ ng/\mu l$$

Results and Conclusions

Table 19: Concentrations of extracted plasmid DNA measured using NanoDrop at 280 nm.

Sample	Concentration [ng/μl]
Nuc Top10 1	11
Nuc Top10 1.1	12.6
Nuc Top10 1 no PB	20.9
Nuc Top10 2	19.5
Nuc Top10 2.1	14.8
Nuc Top10 2 no PB	28.8
Nuc BL21(DE3) 1	96.3
Nuc BL21(DE3) 2	108.5
Nuc BL21(DE3) 3	81.7
Nuc BL21(DE3) 4	79.7
Nuc BL21(DE3) 1 No PB	124.7
Nuc BL21(DE3) 2 No PB	129.9
Nuc BL21(DE3) 3 No PB	93.5
Nuc BL21(DE3) 4 No PB	106.6

Table 20: Concentrations of PCR amplification product, measured using NanoDrop at 280 nm.

Sample	Concentration [ng/μl]
Nuc Top10 1	260.4
Nuc Top10 1.1	231.3
Nuc Top10 1 no PB	102.7
Nuc Top10 2	249.5
Nuc Top10 2.1	138.4
Nuc Top10 2 no PB	73.3

Sufficient concentrations were obtained after the DNA Extraction experiment. When analyzing the gel (Figure 6, the bands in well 2-8 corresponds to a construct of size 1200+ bp. This is not the expected size of around 2800 bp and thus the ligation was unsuccessful.

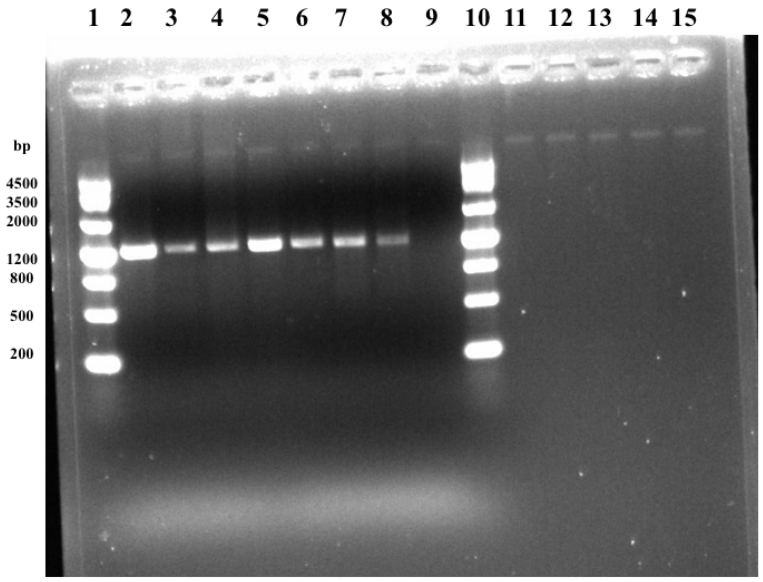

Figure 6: Result of the gel electrophoresis of the PCR amplification product with 1 % agarose at 100 V. (1) DNA Ladder (2) Nuc Top10 1 (3) Nuc Top10 1.1 (4) Nuc Top10 1 no PB (5) Nuc Top10 2 (6) Nuc Top10 2.1 (7) Nuc Top10 2 no PB (8) Positive control (9) Negative control (10) DNA Ladder.

Discussion and Troubleshooting

Since the ligation was successful as cells were growing after transformation, the result of the gel was very misleading. However, when considering the backbone that was used being a digested backbone initially containing the EB-protein (of size 1200 bp), it seems very likely that EB is what is present on the gel. The reason for this could be that EB was not removed from the backbone mixture when digesting it. This resulting in more EB being able to ligate to the backbone and T7 than the amount of Nuc present.

9 Inoculation of pSB1K3-T7-Nuc transformed BL21(DE3) cells in liquid media - second attempt

Responsible

Oscar He and Ellinor Lindholm

Protocols used

Plasmid Extraction
PCR Amplification

Modifications and comments to protocols

DNA Extraction (Qiagen) - Step 1:	centrifugation at 13,000 rpm ($\sim$ 17,000 x g)
DNA Extraction (Qiagen) - Step 10:	DNA elution using Bufer EB
Q5 PCR Amplification:	1 μl DNA sample used in PCR mix.
Q5 PCR Amplification primers:	VF2_R (reverse) VF2_F (forward)

Experimental Setup

Data missing.